

AD \_\_\_\_\_

Award Number: W81XWH-04-1-0648

TITLE: Targeting the MTA 1s-LM04 Pathway in Hormone Resistance

PRINCIPAL INVESTIGATOR: Christopher J. Barnes, Ph.D.

CONTRACTING ORGANIZATION: University of Texas M.D. Anderson Cancer Center  
Houston, TX 77030

REPORT DATE: March 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

|   |                  |                         |                                      |   |  |
|---|------------------|-------------------------|--------------------------------------|---|--|
| <b>REPORT DOCUMENTATION PAGE</b>  |                  |                         |                                      | Form Approved<br>OMB No. 0704-0188                        |  |
| Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b> |                  |                         |                                      |   |  |
| 1. REPORT DATE (DD-MM-YYYY)<br>01-03-2006   |                  | 2. REPORT TYPE<br>Final |                                      | 3. DATES COVERED (From - To)<br>15 JUL 2004 - 14 FEB 2006 |  |
| 4. TITLE AND SUBTITLE<br>Targeting the MTA 1s-LM04 Pathway in Hormone Resistance  |                  |                         |                                      | 5a. CONTRACT NUMBER                                       |  |
|   |                  |                         |                                      | 5b. GRANT NUMBER<br>W81XWH-04-1-0648                      |  |
|   |                  |                         |                                      | 5c. PROGRAM ELEMENT NUMBER                                |  |
| 6. AUTHOR(S)<br>Christopher J. Barnes, Ph.D.<br><br>E-mail: cbarnes@mdanderson.org  |                  |                         |                                      | 5d. PROJECT NUMBER  |  |
|   |                  |                         |                                      | 5e. TASK NUMBER   |  |
|   |                  |                         |                                      | 5f. WORK UNIT NUMBER                                      |  |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br>University of Texas M.D. Anderson Cancer Center<br>Houston, TX 77030  |                  |                         |                                      | 8. PERFORMING ORGANIZATION REPORT NUMBER                  |  |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012   |                  |                         |                                      | 10. SPONSOR/MONITOR'S ACRONYM(S)                          |  |
|   |                  |                         |                                      | 11. SPONSOR/MONITOR'S REPORT NUMBER(S)                    |  |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT<br>Approved for Public Release; Distribution Unlimited  |                  |                         |                                      |   |  |
| 13. SUPPLEMENTARY NOTES   |                  |                         |                                      |   |  |
| 14. ABSTRACT No abstract provided.  |                  |                         |                                      |   |  |
| 15. SUBJECT TERMS<br>No subject terms provided.   |                  |                         |                                      |   |  |
| 16. SECURITY CLASSIFICATION OF:   |                  |                         | 17. LIMITATION OF ABSTRACT<br><br>UU | 18. NUMBER OF PAGES<br><br>3                              | 19a. NAME OF RESPONSIBLE PERSON<br>USAMRMC |
| a. REPORT<br>U  | b. ABSTRACT<br>U | c. THIS PAGE<br>U       |                                      |   | 19b. TELEPHONE NUMBER (include area code)  |

**Re: Grant Award number W81XWH-04-1-0648 entitled, "  
Targeting the MTA1s LMO4 pathway in hormone resistance"  
TARGETING THE MTA1S-LMO4 PATHWAY IN HORMONE RESISTANCE**

**Christopher J. Barnes**

University of Texas M.D. Anderson Cancer Center, Houston, TX

LIM domain transcriptional regulators are critical mediators of pattern formation, organogenesis and cell differentiation. The LIM-only proteins (LMO) consist nearly entirely of two LIM domains and utilize these cysteine-rich, zinc-coordinating regions to help dictate patterns of gene expression and cell fate through mediating protein-protein interactions with DNA binding proteins and transcriptional coregulators. In addition to their developmental roles, LMO proteins may also be critical mediators of cancer development. LMO4, the most divergent LMO protein, was originally cloned from a breast cancer cDNA library and is overexpressed in more than 50% of invasive breast cancers. While investigating the function of a new cytoplasmic protein, MTA1s (metastasis-associated protein 1 (MTA1) short form) in breast cancer, we have found that MTA1s physically interacts with LMO4. Cytoplasmic localization of LMO4 has been noted in late stage human breast cancers. Since MTA1s has been shown to contribute to the cytoplasmic localization of estrogen receptor alpha (ER), enhancement of nongenomic ER signaling, and the development of hormone-resistant breast cancer, we tested the hypothesis that LMO4 is a new ER coregulator that facilitates MTA1s-mediated ER cytoplasmic localization and nongenomic signaling. As a model system, we have developed paired tamoxifen-sensitive and tamoxifen-resistant, ER expressing breast cancer cells that over express LMO4, MTA1s, or both proteins. Routine biochemical, molecular biology and confocal microscopy techniques have been employed in these studies. Results indicate that LMO4 and ER physically interact in vitro and in vivo. Deletion mapping determined that the first 164 amino acids of MTA1s are required for this interaction, while both LIM domains of LMO4 are required for optimal protein-protein interaction. Using transient transfection of an estrogen response element (ERE)-luciferase reporter system, we determined that LMO4 is a potent suppressor of ER-mediated transcriptional activity. Likewise, treatment of ERE-luciferase transfected cells with siRNA directed against LMO4 resulted in a more than four fold increase in both basal and estrogen-induced reporter activity. Although both proteins showed significant cytoplasmic localization under different conditions using immunofluorescent labeling and confocal microscopy, most LMO4-ER colocalization appeared to be in the cell nucleus. Thus LMO4 appears to be an important regulator of ER function. This regulation may encompass both nuclear and cytoplasmic ER. .

*The U.S. Army Medical Research and Materiel Command under W81XWH-04-1-0648 supported this work.*